Hepatic Infection by Thymidine Kinase-Positive and Thymidine Kinase-Negative Herpes Simplex Virus After Partial Hepatectomy

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Herpes simplex virus (HSV) infection of mouse liver after partial hepatectomy was studied. Partial hepatectomy resulted in the rapid onset of cellular DNA synthesis and the appearance of many mitotic figures (peak, 3 days after surgery). Similar changes were not seen in control animals. After partial hepatectomy, the mice were infected with thymidine kinase-positive (TK+) and -negative (TK-) HSV to investigate virus titers in liver tissue during liver cell replication. In control unoperated mice, liver titers of TK+ HSV (2 x 10^3 PFU/g) were greater than those of mice inoculated with TK- HSV (4 x 10^1 to 5 x 10^2 PFU/g). After partial hepatectomy, TK+ and TK- HSV titers increased, and peak TK+ and TK- HSV titers were similar (6 x 10^3 to 8 x 10^5 PFU/g). Hepatic infection was further investigated by infectious center (IC) assays. The numbers of ICs for TK+ HSV increased 50-fold after partial hepatectomy, whereas the increase was less for TK- HSV. From the results of these studies, we hypothesize that the increase in hepatic TK+ HSV after hepatectomy may have been largely due to the increase in ICs, whereas the increase in hepatic TK- HSV was due, in part, to the increase in ICs, but may also have been due to the enhanced synthesis of TK- HSV in replicating liver cells.

The pathogenesis of herpes simplex virus (HSV) is currently an area of great interest. In investigations of experimental HSV infection, we and other workers have provided evidence of the apparent importance of HSV thymidine kinase (TK) expression (4, 10, 14, 15). It had previously been reported that TK-negative (TK-) HSV replicated well in cell culture in dividing cells but not in nondividing cells (7). Based in part on this and on our studies of impaired trigeminal ganglion (neuron) infection by TK- HSV, we hypothesize that in HSV pathogenesis, viral TK expression is important in the establishment of latent neuronal infection (14). To further investigate HSV TK- and TK+ infection in vivo in dividing and nondividing cells, we studied HSV infection in mouse liver cells induced to replicate rapidly by partial hepatectomy.

After the surgical removal of two-thirds to three-fourths of the liver (partial hepatectomy), rapid cell replication and regeneration of the liver ensue (1, 2, 6). In normal unoperated animals, the replication of differentiated hepatocytes (which comprise the great majority of cells in the liver parenchyma) occurs at a low rate, and mitotic figures are not seen. After partial hepatectomy, however, cellular enzymes (1, 3, 9), DNA synthesis (3, 9), and the number of mitotic figures (2, 9) rapidly increase as the liver regenerates. We took advantage of the liver cell replication induced by partial hepatectomy to investigate the in vivo effect of cellular replication on TK+ and TK- HSV infection.

MATERIALS AND METHODS

Viruses, virus inoculation, and isolation. HSV type 1 (HSV-1) strain KOS (TK-), a TK- mutant of this virus (KOS TK-), and the B2006 TK- mutant of S. Kii (Baylor College of Medicine, Houston, Tex.) were grown in primary rabbit kidney cells, and the titration of stocks and isolates was performed in rabbit kidney cells. TK assays were performed on lysates of lysically infected TK- cells as described previously (15). Thymidine (tdR) phosphorylation by the TK- KOS virus was 57 pmol/mg per 20 min; TK- KOS and TK+ B2006 values were 2 and <1% of this, respectively. The mice were inoculated intraperitoneally (i.p.) with 10^5 PFU of virus and killed by exsanguination under ether anesthesia. Liver tissue was removed, and after being washed in Hanks balanced salt solution, approximately 100 mg of tissue was used to make a 10% homogenate in medium containing 10% serum. After clarification, supernatants were titrated under 0.5% methylcellulose medium. From infectious center (IC) assays, liver was collected as described above, and
after trypsinization, filtration, and cell counting, suspensions of 10^6 cells per ml were titrated.

**Animals and methods of partial hepatectomy.** Five-to seven-week-old random-bred male and female CD-1 mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were anesthetized with sodium pentobarbital. Partial hepatectomy was performed with slight modification by the method of Higgins and Anderson (6). The left lateral and median lobes, constituting approximately two-thirds of the total liver, were ligated and excised, leaving the right lateral and caudate lobes. Sham hepatectomy was performed with similar exposure of the liver but without removal of any liver tissue.

**Preparation of liver cell DNA.** To measure liver cell DNA synthesis during hepatic regeneration, we injected hepatectomized and control mice i.p. with 40 μCi of [3H]TdR (80.1 Ci/mmol; New England Nuclear Corp., Boston, Mass.) 1 h before sacrifice. Liver tissues were collected and washed in phosphate-buffered saline, and approximately 600 mg was used for cellular DNA extraction. DNA was extracted by the method of Marmur (11). Briefly, minced tissues were homogenized in 0.05 M Tris (pH 7.4) containing 0.005 M magnesium acetate and 0.04 M sodium chloride. After centrifugation (12,000 × g for 5 min), nuclei were suspended in Tris buffer (pH 8.0) containing 0.15 M sodium chloride and 0.01 M EDTA and lysed with 1% sodium dodecyl sulfate. After extraction in Tris-buffer-saturated phenol-chloroform-isoamyl alcohol (12:12:1), DNA was spooled with the careful addition of 100% ethanol. DNA was redissolved in 0.15 M sodium chloride-0.015 M sodium citrate buffer and incubated with RNase A (100 μg/ml; Worthington Diagnostics, Freehold, N.J.) and RNase T1 (100 U/ml; Sigma Chemical Co., St. Louis, Mo.). After repeated phenol extraction and precipitation, DNA concentrations were estimated by optical density at 260 nm, and the radioactivity of samples was measured in a scintillation counter.

**RESULTS**

**Regeneration of liver after partial hepatectomy.** After an approximately two-thirds partial hepatectomy, the incorporation of [3H]TdR into DNA, numbers of hepatic mitotic figures, and the weight of regenerating liver were calculated. The incorporation of [3H]TdR into hepatic DNA rapidly increased after hepatectomy; a more than 100-fold increase in [3H]TdR was noted, with the peak occurring 72 h after surgery (Fig. 1A). In normal control mice (unoperated controls), [3H]TdR incorporation was 13% of the value obtained for partially hepatectomized animals sacrificed after 24 h and 2% of those sacrificed after 72 h. In operated control mice in which the abdominal wall and peritoneum were opened and then closed (sham-hepatectomized controls), [3H]TdR incorporation 24 h postsurgery was 12% of that for partially hepatectomized animals and was therefore similar to the control unoperated value. Limited [3H]TdR incorporation in these control groups supports the conclusion that [3H]TdR incorporation into DNA was for the most part specific for hepatic regeneration.

Hepatic regeneration after partial hepatectomy was also apparent from measurements of liver weights (Fig. 1B). The rate of liver weight increase was rapid for the first 4 days after partial hepatectomy and somewhat less rapid during the next 4 days. By 8 days after hepatectomy, liver weight (mean, 1.1 g) was slightly less

![Graph A](http://iai.asm.org/)

![Graph B](http://iai.asm.org/)

**FIG. 1.** (A) [3H]TdR incorporation into liver cell DNA at different times after partial hepatectomy. [3H]TdR was injected into all mice at 1 h before sacrifice. [3H]TdR incorporation into hepatic DNA of unoperated control mice is shown by the dashed line. The data are the means and standard errors, with three to four mice per time point. (B) Regeneration of liver weight after partial hepatectomy. The liver weight of unoperated control mice is shown by the dashed line. The data are the means and standard errors, with three mice per time point.
than that of normal unoperated mice (mean, 1.4 g).

As a third index of liver regeneration, the number of mitotic figures in hepatic cells was studied after partial hepatectomy. For counts of mitotic figures, 1,000 cells from liver sections of two mice at each time point were counted. In control unoperated mice no mitotic figures were noted, and no mitotic figures were noted in liver sections immediately after or 12 h after surgery (Fig. 2A). One day after surgery, mitotic figures were noted in 1.3% of hepatocytes, and the percentage increased to 6.3 and 6.4%, respectively, 2 and 3 days after surgery. Mitotic figures 3 days after partial hepatectomy are shown in Fig. 2B. The percentage of mitotic figures decreased markedly by 4 days postsurgery, and by 8 days the percentage was 0.2%. In summary, the regeneration studies indicated hepatic regeneration with a peak of DNA synthetic activity in regenerating hepatocytes 2 to 3 days after partial hepatectomy.

**TK**<sup>+</sup> HSV infection in liver after partial hepatectomy. HSV titers were measured in liver homogenates of animals that had undergone partial hepatectomy and of controls (sham-hepatectomized mice and unoperated mice). HSV was injected i.p. at various times after surgery, and hepatic HSV titers were determined in mice sacrificed 24 h later. In preliminary studies, we observed that after i.p. inoculation, the liver titers of HSV-1 increased by up to 0.5 log U between 1 and 24 h, remained fairly constant to 48 h, and decreased by 72 h (Tenser, unpublished data). At 24 h after the inoculation of TK<sup>+</sup> HSV, virus titers in sham-hepatectomized mice, over several time points, were similar to the mean titer in unoperated mice. However, virus titers in partially hepatectomized mice markedly increased, with a peak titer at 60 h after surgery. The mice in the 60-h virus group were inoculated with virus at 36 h after partial hepatectomy, a time when liver cell DNA synthesis was rapidly increasing. The 60-h titer for the partial-hepatectomy group was more than 2 log units greater than that of the two control groups. HSV titers decreased on days 4 and 10 post-hepatectomy, but were still above control values.

**TK**<sup>−</sup> HSV infection in liver after partial hepatectomy. After the i.p. inoculation of TK<sup>−</sup> KOS virus, the hepatic titers of unoperated control and sham-hepatectomized mice were similar (Fig. 3B). The titers were approximately 100-fold less than those in TK<sup>+</sup>-infected mice (Fig. 3A). After partial hepatectomy, we observed a marked increase in the titers of mice sacrificed 60 h after surgery; the average titer was approximately 4 log U greater than the average titer in control mice. In addition, the 60-h KOS TK<sup>−</sup> titer (8.3 × 10<sup>5</sup> PFU/g; Fig. 3B) was almost identical to the 60-h TK<sup>+</sup> titer (7.7 × 10<sup>5</sup> PFU/g; Fig. 3A). In unoperated and sham-hepatectomized control mice infected with B2006 TK<sup>−</sup>-HSV, the hepatic titers were intermediate between the titers of KOS TK<sup>+</sup> and KOS TK<sup>−</sup> viruses (Fig. 3C). The B2006 TK<sup>−</sup> titers were similar for mice in both control groups. The mean hepatic virus titer at 60 h after partial hepatectomy was more than 2 log U greater than control values and was similar to the titers of mice infected with the KOS TK<sup>+</sup> and KOS TK<sup>−</sup> viruses.

**HSV IC titers after partial hepatectomy.** As indicated above, after partial hepatectomy, increases in TK<sup>+</sup> and particularly in TK<sup>−</sup> HSV titers were noted in liver homogenates. To further investigate these increases, the numbers of ICs were determined in mice infected with KOS TK<sup>+</sup> or KOS TK<sup>−</sup> viruses. Control unoperated, control sham-hepatectomized, and partially hepatectomized mice were studied. The animals were inoculated i.p. with virus at 36 h after surgery, and liver cell suspensions for IC titrations were prepared 1 or 24 h later. Somewhat surprisingly, 1- and 24-h IC titers were very similar for individual treatment groups (Table 1). The ICs in unoperated TK<sup>−</sup> HSV-infected mice were approximately 10-fold less than those in TK<sup>−</sup>-infected mice. A similar TK<sup>+</sup>/TK<sup>−</sup> difference was observed in sham-hepatectomized mice. After partial hepatectomy, the average number of TK<sup>−</sup> ICs (7 of 10,000 liver cells) increased in comparison to sham-hepatecromized control (<0.6 of 10,000 cells) and unoperated control (<0.3 of 10,000 cells) values. The

### TABLE 1. HSV ICs after partial hepatectomy, sham hepatectomy, and no treatment<sup>a</sup>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. (mean ± SEM)&lt;sup&gt;b&lt;/sup&gt; of ICs per 10&lt;sup&gt;6&lt;/sup&gt; liver cells after i.p. virus inoculation with:</th>
<th>TK&lt;sup&gt;−&lt;/sup&gt; HSV</th>
<th>TK&lt;sup&gt;+&lt;/sup&gt; HSV</th>
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<tbody>
<tr>
<td>None</td>
<td>3.7 ± 0.6</td>
<td>&lt;0.3 ± 0.09</td>
<td></td>
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<tr>
<td>Sham hepatectomy</td>
<td>3.5 ± 0.8</td>
<td>&lt;0.6 ± 0.03</td>
<td></td>
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<tr>
<td>Partial hepatectomy</td>
<td>201 ± 14</td>
<td>7.0 ± 3.4</td>
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<sup>a</sup> Partial hepatectomy and sham surgery were performed 36 h before the i.p. inoculation of KOS TK<sup>−</sup> or TK<sup>−</sup> HSV (10<sup>5</sup> PFU, four to five mice per condition). The animals were sacrificed 1 or 24 h later, and after trypsinization of liver, the numbers of infected cells were determined. The differences between 1- and 24-h IC values were less than 0.3 log U, and these results were pooled.

<sup>b</sup> Values less than 1.0 were averages, including assays in which the absence of ICs was scored as zero. P < 0.05 by the Student t-test for the comparison of TK<sup>−</sup> HSV after partial hepatectomy with other TK<sup>−</sup> HSV groups and of TK<sup>−</sup> with TK<sup>−</sup> after partial hepatectomy.
average number of TK\textsuperscript{+} ICs after partial hepatectomy (200 of 10,000 cells) increased 50-fold compared with IC titers in the sham-hepatectomized and unoperated control groups (4 of 10,000 cells); this increase was similar to that in hepatic cell-free virus titers (Fig. 3A). TK\textsuperscript{-} ICs after partial hepatectomy increased more than 10-fold compared with controls, but the increase
Days after partial hepatectomy

A

B

C

FIG. 3. HSV titers in mouse liver homogenates after partial hepatectomy (X), in sham-hepatectomized controls (●), and in unoperated controls (○). In all cases, the mice were sacrificed at 24 h after i.p. inoculation of virus. The means and standard errors are given, with three mice per time point. (A) Titers after TK⁺ KOS HSV inoculation. (B) Titers after TK⁻ KOS HSV inoculation. (C) Titers after B2006 TK⁻ HSV inoculation.
was much less than that in cell-free virus in similar mice.

DISCUSSION

HSV titers in liver homogenates at 24 h after i.p. infection were enhanced in mice in which a partial hepatectomy had been performed. The virus titers in liver were highest in mice infected 36 h after partial hepatectomy (sacrificed at 60 h). Although we did not test a large number of time points after hepatectomy, the liver homogenate titers of mice infected before or after 36 h postsurgery were tested; the titer for the 36-h group was considerably higher than that for the other groups. Because the liver regenerated subsequent to partial hepatectomy and because cell-free virus was measured in a constant weight of liver, it is possible that for later time points and in control mice, the amount of virus was slightly diluted by the larger amount of liver present. This is an unlikely explanation of results, however, because the increase in the liver weight was slight in comparison with the virus titer changes. In addition, this would not explain the higher titer for the 36-h group (sacrificed at 60 h) compared with the 12-h group (sacrificed at 36 h).

In the present experiment, the peak time for $[^3]H$Tdr incorporation into cellular DNA was 2 to 3 days after partial hepatectomy. This was somewhat later than the peak time reported by Vincent et al. (16). The initial rapid increase in $[^3]H$Tdr incorporation into DNA that we observed occurred 6 h later than that noted by Church and McCarthy (3). Our observation of a peak of mitotic figures 2 to 3 days after partial hepatectomy is in accord with that time period being one of markedly increased DNA synthesis. The 2- to 3-day peak is similar to the 3-day peak reported by Wilson et al. after partial hepatectomy in mice (17), although other investigators reported a peak of mitoses 28 to 30 h after surgery (2). The reasons for these differences are not apparent, although the ages of the animals and the amounts of liver excised influence the rates of regeneration (3). Despite differences between investigations, partial hepatectomy appears to be a useful method for the in vivo induction of cell replication. The increased HSV titers noted in liver during the period of regeneration may be related to cellular DNA synthesis, although increased cellular RNA synthesis and the effects of other aspects of the dividing cells cannot be ruled out.

It was apparent that both TK$^+$ and TK$^-$ HSV replicated to high titers in regenerating liver. Although TK$^+$ titers in liver homogenates from control mice were lower than TK$^-$ titers, TK$^+$ and TK$^-$ titers in mice infected 36 h after partial hepatectomy were very similar. The enhanced titers of TK$^-$ HSV in regenerating liver are in accord with the observation that TK$^-$ HSV replicates well in vitro in dividing, but not in nondividing, cells (7). The increased TK$^-$ titers in comparison with control values support our hypothesis that in vivo HSV TK expression may be important for the infection of nondividing neurons but is less important for the infection of replicating cells (14). Enhanced TK$^+$ HSV replication in regenerating liver cells may be similar to the HSV infection of stimulated lymphocytes (5, 8, 13). It would be of interest to perform similar studies of hepatic infection by TK$^+$ and TK$^-$ HSV-2 since in normal mouse liver HSV-2 replication is greater than HSV-1 (12).

Somewhat surprisingly, the numbers of liver cell ICs increased greatly during the post-hepatectomy period. The magnitude of the increase in ICs for TK$^-$ HSV was similar to that in cell-free liver homogenate titers during the same time period. However, the magnitude of the cell-free virus increase for TK$^-$ HSV was much greater than the increase in ICs. From the magnitude and timing of the increased TK$^+$ and TK$^-$ cell-free titers and the increase in ICs, it may be hypothesized that the increased TK$^+$ titers were largely due to an increase in ICs and that the increased TK$^-$ titers were due, in part, to increased ICs and also to enhanced virus replication in the regenerating liver cells.

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LITERATURE CITED


